

Fucosyltransferase VII-positive, skin-homing T cells in the blood and skin lesions of atopic dermatitis patients

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Abstract: Patients with atopic dermatitis (AD) have an abnormally increased frequency of cutaneous lymphocyte antigen (CLA)⁺ Th2 cells responsible for local inflammation; however, this is paradoxical, given the well-recognized defective capacity of Th2 cells to migrate to the skin sites of inflammation. These discrepant observations would stem from the ambiguity of CLA⁺ T cells, because CLA does not represent the epitope required for binding to E-selectin but the epitope generated by fucosyltransferase VII (Fuc-TVII) and because skin-homing T cells are composed of three distinct subpopulations; Fuc-TVII⁺ E-selectin ligand (ESL)⁺ CLA⁺, Fuc-TVII⁺ ESL⁺ CLA⁺ and Fuc-TVII[−] ESL[−] CLA⁺ cells. We therefore asked which subpopulations of skin-homing Th2 cells could be increased in the blood and skin lesions of AD. We

analysed the frequencies of the three subpopulations in purified CD4⁺ peripheral blood T cells from AD patients and healthy controls by immunohistochemistry and flow cytometry. The Fuc-TVII⁺ CLA⁺ or CLA⁺ ESL⁺ CCR4⁺ cells were dramatically increased in frequency not only in the blood but also in the skin lesions of AD patients and this increase was related to the severity of the clinical symptoms. Our data indicate the clinical importance of identifying skin-homing T cells with the potent capacity to migrate into the skin by analysing their Fuc-TVII expression and E-selectin binding ability in patients with AD.

Key words: atopic dermatitis – lymphocyte antigen – fucosyltransferase VII – E-selectin ligands – cutaneous Th2 cells

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Introduction

Selective migration of memory T cells to sites of inflammation is a multistep process controlled by the interaction of specific receptor–ligand pairs (1–6). Expression of the cutaneous lymphocyte antigen (CLA) recognized by monoclonal antibody (mAb) HECA-452 (4,5) on distinct subsets of T cells was generally thought to contribute to their skin homing by interacting with E-selectin on endothelial cells (6,7). Recent studies including our own have shown, however, that the CLA epitope is a post-translational modification of the protein core of P-selectin glycoprotein-1 (PSGL-1) by the activity of $\alpha(1,3)$ -fucosyltransferase VII (Fuc-TVII) but does not necessarily represent the epitope required for binding to E-selectin (8–13). However, most of the previous studies performed on skin-homing T cells were not extended beyond their expression of CLA because of the lack of mAb by which expression of Fuc-TVII at the protein level can be detected. In this regard, we have recently established a reliable immunohistochemical assay that allows the identification of Fuc-TVII expression in freshly prepared T cells and T cell populations in tissue

sections (11,12). With the use of this assay, we have recently demonstrated that differentiation of naïve T cells into the skin-homing memory subsets capable of binding E-selectin is critically dependent upon the induction of Fuc-TVII activity but not CLA expression and that skin-homing CD4⁺ T cells are composed of three distinct subpopulations representing different stages of differentiation, the early activated Fuc-TVII⁺ CLA[−], the late activated Fuc-TVII⁺ CLA⁺ and the terminally differentiated Fuc-TVII[−] CLA⁺ cells (11–13).

Atopic dermatitis (AD) is generally perceived as a Th2-dominated disease and many of the characteristics of skin inflammation can be attributed to Th2 cell-derived cytokines (14–16). According to recent studies, patients with AD have an abnormally increased frequency of CLA⁺ T cells capable of producing Th2 cytokines in their circulation (16,17). Recent studies, however, paint a paradoxical picture in which Th2 cells have been shown to be defective in their capacity to migrate to sites of inflammation because of their intrinsically lower capacity to bind E-selectin that stems from lower expression of Fuc-TVII (18,19). It is also unknown to what extent T-cell measurements in

the blood reflect the overall status of the immune system. Therefore, it could be alternatively interpreted as indicating that the increased CLA⁺ Th2 cells in AD are of the Fuc-TVII⁺ CLA⁺ phenotype that has a greatly diminished capacity to bind E-selectin, thereby accumulating in the blood. We therefore explored whether increased subsets of T cells expressing CLA in the blood from patients with AD indeed represent those with the late activated phenotype capable of binding E-selectin, and whether these phenotypic properties could be also specifically detected in the corresponding T cells in the skin lesions of AD. We report herein that Fuc-TVII⁺ CLA⁺ Th2 cells with the most potent capacity to bind E-selectin are dramatically increased in the blood and that this increase is related to the severity of clinical symptoms. Our data show that the frequencies of this population can be a useful marker for monitoring and predicting relapse in patients with AD.

Materials and methods

Reagents

For FACS (flow cytometry) analysis, anti-CD4 mAb (allophycocyanin-labelled) and anti-CCR4 mAb (PE (phycoerythrin)-labelled) were purchased from BD Pharmingen (San Diego, CA, USA) and goat F(ab')₂ anti-human immunoglobulin G (IgG) Fc mAb (PE-labelled) were purchased from Immunotech (Marseilles, France), anti-CXCR3 mAb (carboxyfluorescein succinimidylester-labelled) was purchased from Dako (Glostrup, Denmark), anti-CD33 was purchased from Becton Dickinson (Mountain View, CA, USA) and anti-CLA mAb (HECA-452, rat IgM, FITC-labelled) was a gift from L. Picker (4). For immunohistochemical analysis, mAb against CD3 (Beckton Dickinson) and CD4 (Nichirei, Tokyo, Japan) were purchased commercially and anti-Fuc-TVII enzyme mAb (KM1939, mouse IgG) was prepared in our laboratory, as described previously (11,12).

Patients and preparation of peripheral blood mononuclear cells

Nine patients (age, 21–36 years; median age, 30.2 years) with adult type AD according to the criteria of Hanifin and Rajka (20) were enrolled in this study. None of them had asthma or other atopic conditions. They never received systemic corticosteroids or psoralen-ultraviolet A therapy, or any systemic therapy for at least 6 months before study entry. The severity of the disease was clinically assessed as mild (3,4), moderate (4.5–7.5) and severe (8,9), according to the method of Rajka and Langeland (21). The mean severity score of patients enrolled in this study was 7.7. After informed consent was obtained from these AD patients, peripheral blood mononuclear cells (PBMC) were isolated by density gradient sedimentation over Ficoll-Hypaque (Sigma Chemicals Co., St. Louis, MO, USA). PBMC

were resuspended in the medium, as appropriate, for further purifications. CD4⁺ T cells were isolated from the PBMC preparations by negative selection on magnetic separation using a CD4⁺ T cell isolation Kit (MACS; Miltenyi Biotec, Bergish Gladbach, Germany) according to the manufacturer's protocol. The isolated CD4⁺ samples were highly enriched for CD4⁺ T cells: recovered CD4⁺ T cells showed >91.0% CD3⁺/CD4⁺ purity as assessed by flow cytometry. Of particular importance to our studies, the isolated CD4⁺ T cell preparations contained in all instances no or very few (<1%) monocytes as evaluated by morphology and CD33 expression. Six healthy blood donors without experience of allergic symptoms (age, 22–39 years; median age, 29.4 years) were selected as normal controls.

Preparation of tissue specimens

Skin biopsy specimens were obtained from five AD patients (age, 20–33 years; median age, 29.0 years) and five psoriatic patients (age, 22–38 years; median age, 28.6 years). These biopsies were taken from typical chronic lesions on their trunk or extremities. The biopsy specimens were frozen immediately in liquid nitrogen, embedded in ornithine carbomoyl transferase compound (Tissue Tek II; Miles Laboratories, Naperville, IL, USA) and stored at –80°C until use.

Detection of Fuc-TVII and CLA or CD3 expression on T cells

Immunohistochemical staining of Fuc-TVII and CLA or CD3 was performed on purified CD4⁺ T cell preparations, and tissue specimens, as previously described (11,12). Briefly, the cells were centrifuged onto microscope slides using a Sakura autosmear CF12D (Sakura Seiki Co., Tokyo, Japan), air-dried and stored at –80°C until use. In case of tissue specimens, frozen sections 6-μm thick were cut on a cryostat, air-dried and stored –80°C. Slides fixed with 4% paraformaldehyde (PFA) were incubated with Fuc-TVII-specific mAb, and were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase. For efficient intracellular staining of Fuc-TVII, a 37° or 50°C temperature treatment in 5% zinc sulphate for 10 min was required for cell specimens or tissue specimens, respectively. The activity of the primary antibody was revealed by incubating with biotinylated horse anti-mouse IgG (Vector Laboratories, Inc., Burlingame, CA, USA) in the same diluent and streptavidin/biotinylated horseradish peroxidase complex (Dako), followed by the visualization of peroxidase activity using AEC (3-amino-9-ethyl carbazole) (Dako) as chromogen. The slides were counterstained with Mayer hematoxylin. Negative controls were incubated with isotype-matched mouse mAb of irrelevant specificity or phosphate buffer saline (PBS), instead of the primary mAb. For double immunohistochemical staining, the first staining step

(Fuc-TVII) with the aforementioned protocol was used, and this was followed by the second step (CLA or CD3) in which avidin-biotin-alkaline phosphatase complex (ABC-AP) and Vector blue detection system (Vector Laboratories) were used, as described previously (11,12).

Flow cytometric analysis

Detection of E-selectin ligand (ESL) expression was performed on purified CD4⁺ T cell preparations by flow cytometry as previously described (13). Briefly, 1×10^5 cell suspensions of purified CD4⁺ T cells were incubated with 10 $\mu\text{g}/\text{ml}$ of rE-selectin-IgG Fc chimera (R & D Systems, Minneapolis, MN, USA) in an incubation medium (3% fetal calf serum in HBSS [Hank's Buffered Salt Solution]) for 30 min at 37°C. After washing, the cells were stained with goat F(ab')₂ anti-human IgG Fc mAb (PE-labelled or FITC-labelled), anti-CLA mAb (FITC-labelled), anti-CCR4 mAb (PE-labelled), and anti-CXCR3 (CFS (carboxy-fluorescein succinimidylester)-labelled) for 30 min at 4°C. Stained cells were fixed with 1% PFA in PBS and analysed using a FACS Calibur flow cytometer (BD Biosciences, Mountain View, CA, USA). Controls were incubated with 10 $\mu\text{g}/\text{ml}$ of human IgG Fc (ICN Pharmaceuticals, Costa Mesa, CA, USA).

Quantification of stained cells

Immunohistochemically stained sections were assessed using an Olympus BH-2 microscope in a blind fashion by two observers (Yoshiko Mizukawa and Yoshimi Yamazaki) independently. For each specimen at least five randomly selected fields of areas were assessed under 400 $\times$ magnification. An average of 500 cells was counted per slide and the percentage of positively stained cells was calculated. Double-stained cells were characterized if both red and blue colours could be discerned within one cell. For FACS, data were analysed by ANOVA for repeated measures for within-group comparisons and one-way ANOVA for comparisons among groups.

Results

Fuc-TVII⁺ CLA⁺ T cells capable of binding E-selectin are increased in AD

We initially compared frequencies of three subpopulations of skin-homing T cells, Fuc-TVII⁺ CLA⁻, Fuc-TVII⁺ CLA⁺ and Fuc-TVII⁻ CLA⁺ cells (11–13), in purified CD4⁺ T cells of AD patients with those in healthy controls. We found that the terminally differentiated Fuc-TVII⁻ CLA⁺ T cells were the most abundantly identifiable population in controls; while late activated Fuc-TVII⁺ CLA⁺ cells were the most, followed by Fuc-TVII⁻ CLA⁺ cells in AD patients (Fig. 1a). When AD patients were subdivided into two groups, severe ($n = 5$) and mild/moderate ($n = 4$), as described previously (21,22), frequencies of total Fuc-TVII⁺

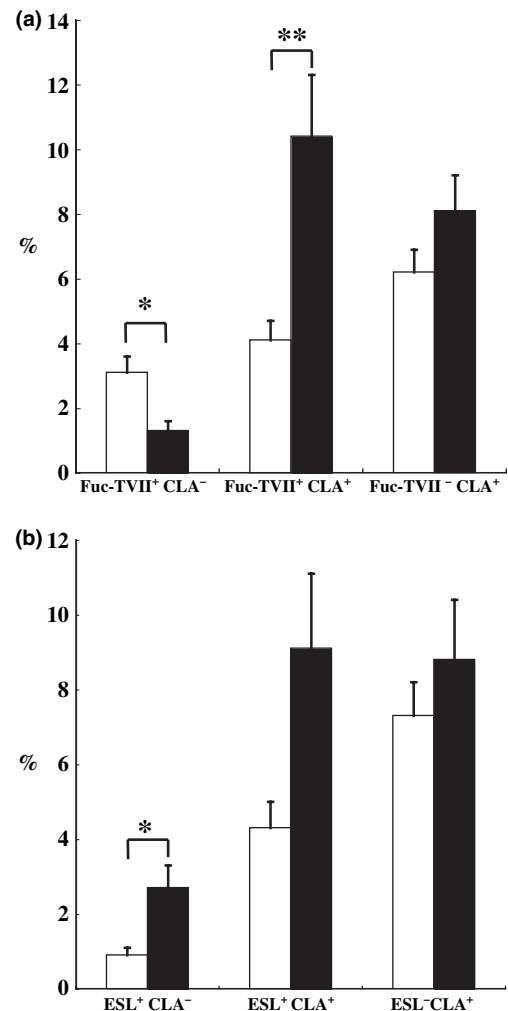


Figure 1. Comparison of the frequencies of Fuc-TVII⁺ CLA⁻, Fuc-TVII⁺ CLA⁺ and Fuc-TVII⁻ CLA⁺ T cells (a) and of those of ESL⁺ CLA⁻, ESL⁺ CLA⁺ and ESL⁻ CLA⁺ T cells (b) in CD4⁺ T cells from atopic dermatitis (AD) patients and healthy subjects. Nine patients with AD (closed bars) and six healthy controls (open bars) were analysed by immunohistochemical staining (a) and flow cytometry (b), as described in Materials and methods. Results are expressed as the mean percentage of each subset ± SEM in CD4⁺ T cells. Statistical significance between the two groups using Student's *t*-test is indicated: * $P < 0.05$; ** $P < 0.01$.

($16.2 \pm 2.2\%$ vs. $4.2 \pm 1.3\%$; $P < 0.005$) and CLA⁺ T cells ($24.6 \pm 1.6\%$ vs. $7.9 \pm 1.0\%$; $P < 0.005$) and the Fuc-TVII⁺ CLA⁺ subset ($14.7 \pm 2.0\%$ vs. $3.2 \pm 1.0\%$; $P < 0.005$) were significantly higher in the severe group than in the mild/moderate group. In particular, the frequency of the Fuc-TVII⁺ CLA⁺ subset was most positively correlated with the severity of the disease.

We next investigated whether the Fuc-TVII⁺ CLA⁺ phenotype could be endowed with the capacity to most efficiently bind E-selectin. However, it was not technically possible to test this possibility by directly measuring the

capacity of this Fuc-TVII⁺ CLA⁺ CD4⁺ subset to bind E-selectin. We alternatively tested this possibility by measuring the capacity to bind E-selectin of CLA⁺ CD4⁺ T cells freshly purified from AD patients, because our previous study of PBMC from healthy controls indicates that Fuc-TVII⁺ CLA⁺ CD4⁺ T cells were close to CLA⁺ CD4⁺ T cells able to bind E-selectin in frequency and timing of the appearance during *in vitro* differentiation cultures from naïve T cells (13). Our flow cytometric analysis showed that the frequencies of ESL⁺ CLA⁺ CD4⁺ T cells in AD patients increased twofold in parallel with the increase in those of the Fuc-TVII⁺ CLA⁺ CD4⁺ subset as compared with those of the corresponding population in healthy controls (Figs 1b and 2a,b): the frequencies of ESL⁺ CLA⁺ CD4⁺ T cells were close to those of Fuc-TVII⁺ CLA⁺ CD4⁺ T cells ($9.1 \pm 2.0\%$ vs. $10.4 \pm 1.9\%$) in AD. There was also a good correlation between the increase in the frequencies of the ESL⁺ CLA⁺ subset and the severity of the disease (severe versus mild/moderate: $13.6 \pm 2.2\%$ vs. $4.6 \pm 0.2\%$; $P < 0.01$). These results indicate that CD4⁺ T cells with the potent capacity to bind E-selectin are increased in frequency in the blood of AD patients. Thus, preferential accumulation of CLA⁺ CD4⁺ T cells in the circulation of AD patients is unlikely to be because of defects in their capacity to bind E-selectin.

To further confirm the relationship between the increase in the ESL⁺ CLA⁺ subset and the severity of clinical symptoms, AD patients' CD4⁺ T cells were phenotypically analysed before and after treatment with topical corticosteroids. Flow cytometric analysis showed increased frequencies of the ESL⁺ CLA⁺ in circulation when their disease was active (Fig. 2b,c); as shown in a representative case (Fig. 2c,d), symptomatic flares were consistently accompanied by the increase in this population and improvement in symptoms mirrored the reduction.

Increased Fuc-TVII⁺ CLA⁺ T cells are Th2 cells

Ideally, to determine whether increased Fuc-TVII⁺ CLA⁺ T cells or ESL⁺ CLA⁺ T cells in CD4⁺ T cells from AD patients are of the Th2 phenotype, the ability of this population to produce Th2 cytokines should be examined. This approach, however, was not applicable to the determination of the phenotype, because of the small number of ESL⁺ CLA⁺ CD4⁺ T cells capable of producing Th2 cytokines upon stimulation *in vitro*. In this regard, recent studies have shown that 90% of CCR4⁺ CD4⁺ T cells in humans are of the Th2 phenotype (23–25) while CXCR3 is preferentially expressed on Th1 cells (24,25); and that CCR4⁺ CD4⁺ T cells are increased in both peripheral blood and skin lesions of AD (26,27). We therefore investigated expression of CCR4 on the ESL⁺ CD4⁺ T cell population in AD patients and healthy controls, to determine whether increased Fuc-TVII⁺ CLA⁺ T cells or ESL⁺ CLA⁺ T cells in

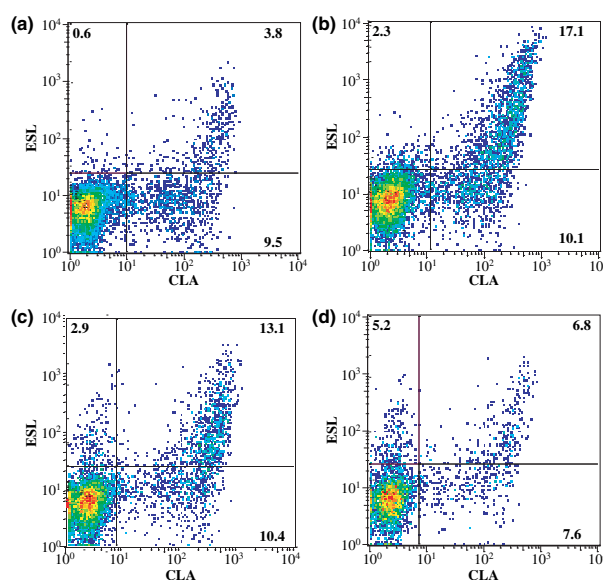


Figure 2. Flow cytometric detection of E-selectin ligand (ESL) and cutaneous lymphocyte antigen (CLA) expression by purified CD4⁺ T cells obtained from a healthy control and representative atopic dermatitis (AD) patients. The numbers in each quadrant indicate the per cent of positive cells. (a) A healthy control. (b) An AD patient at the active stage. (c) Another AD patient at the active stage before treatment. (d) The same patient as (c) after treatment. Similar results were obtained in three independent experiments using samples from two additional individuals.

CD4⁺ T cells from AD patients are of the Th2 phenotype. Based upon the expression of CCR4 and CLA, three subsets of CD4⁺ T cells, CCR4[−] CLA⁺, CCR4⁺ CLA⁺ and CCR4⁺ CLA[−], can be identified. On average, $16.0 \pm 3.6\%$ of CD4⁺ T cells were CCR4⁺ CLA⁺, $13.7 \pm 0.9\%$ were CCR4⁺ CLA[−] and $2.1 \pm 0.2\%$ were CCR4[−] CLA⁺ in the AD patients ($n = 9$). These results indicate that the vast majority (>88%) of CLA⁺ CD4⁺ T cells express CCR4 in AD patients. As shown in representative cases (Fig. 3), CCR4⁺ CD4⁺ T cells capable of binding E-selectin were increased in frequency in AD patients, as compared with healthy controls; the mean percentage of CCR4⁺ CD4⁺ T cells that bind E-selectin was significantly higher in AD patients than that of the corresponding T cells in healthy controls ($44.4 \pm 5.2\%$ vs. $19.4 \pm 1.5\%$; $n = 6$; $P < 0.005$). In contrast, there was no significant difference in the capacity to bind E-selectin of CXCR3⁺ CD4⁺ T cells between AD patients and healthy controls ($19.8 \pm 2.6\%$ vs. $14.8 \pm 2.8\%$). These results indicate that many if not all of the Fuc-TVII⁺ CLA⁺ or ESL⁺ CLA⁺ CD4⁺ T cells, which are increased in frequency in the blood of AD patients, are of the Th2 phenotype.

In addition, we found improvement in symptoms by the successful symptomatic treatment to be consistently accompanied by the decrease in the frequencies of CCR4⁺ ESL⁺

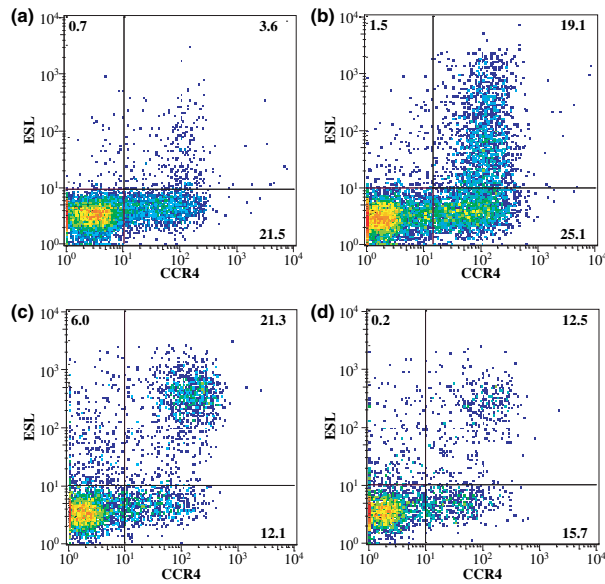


Figure 3. Flow cytometric detection of E-selectin ligand (ESL) and CCR4 expression by purified CD4⁺ T cells obtained from a healthy control and representative atopic dermatitis (AD) patients. (a) A healthy control. (b) An AD patient at the active stage. (c) Another AD patient at the active stage before treatment. (d) The same patient as (c) after treatment. Similar results were obtained in three independent experiments using samples from two additional individuals.

CD4⁺ T cells (Fig. 3c vs. d), as shown in ESL⁺ CLA⁺ CD4⁺ T cells (Fig. 2c vs. d). In contrast, no significant reduction was detected in the frequencies of CCR4⁺ ESL⁺ CD4⁺ T cells upon the improvement in clinical symptoms. In parallel to the decrease in the frequencies of CCR4⁺ ESL⁺ CD4⁺ T cells, the frequencies of Fuc-TVII⁺ CLA⁺ CD4⁺ T cells also fell upon the improvement in this representative case (from 30.1% to 5.6%). These results suggest that CCR4⁺ ESL⁺ Fuc-TVII⁺ CD4⁺ T cells would be the key cells responsible for the effector functions of CD4⁺ T cells in deteriorating AD.

Increased frequency of Fuc-TVII⁺ CLA⁺ T cells in AD lesions

We finally asked whether the increase in Fuc-TVII⁺ CLA⁺ T cells in the blood could be reflected in their increased appearance in the skin lesions. In addition, to determine whether our findings are general results of skin inflammation or are an AD-specific phenomenon, we extended our study to analyse five cases of psoriasis, together with five cases of AD.

Double immunohistochemical staining showed that the Fuc-TVII enzyme was detected in the cytoplasm of CD3⁺ or CLA⁺ expressing cells (Fig. 4). The most remarkable difference between AD and psoriasis was noted in the frequency of Fuc-TVII⁺ CLA⁺ T cells within the epidermis and dermis; in particular, as compared with those in

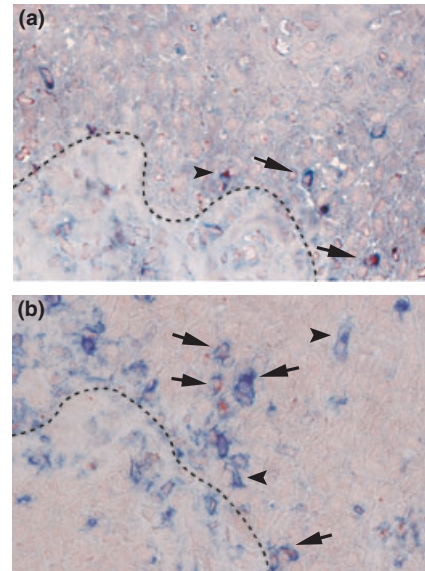


Figure 4. Double immunohistochemical detection of fucosyltransferase VII (Fuc-TVII) and cutaneous lymphocyte antigen (CLA) expression by infiltrating T cells in biopsy specimens obtained from atopic dermatitis (AD) lesions (original magnification $\times 132$). (a) Scattered T cells predominantly detected in the epidermis, co-express surface CD3 (blue) and cytoplasmic Fuc-TVII (red). Fuc-TVII⁺ CD3⁺ cells are indicated by arrows. Fuc-TVII⁺ CD3[−] cells are indicated by arrowheads. Dotted line shows the basement membrane zone. (b) Most of the CLA-positive cells (blue) within the epidermis co-express Fuc-TVII (red). Fuc-TVII⁺ CLA⁺ cells are indicated by arrows. Fuc-TVII[−] CLA⁺ cells are indicated by arrowheads. Dotted line shows the basement membrane zone.

psoriasis lesions, in which Fuc-TVII⁺ T cells were relatively restricted to the epidermal component, Fuc-TVII⁺ CLA⁺ T cells were also abundantly detected even in the dermis of AD lesions (Table 1). These results indicate that Fuc-TVII⁺ CLA⁺ T cells, the most abundantly identifiable population in the blood of AD patients, are also increased in the skin lesions of AD.

Discussion

In the experiments presented here, we have shown that Fuc-TVII⁺ CLA⁺ T cells able to efficiently bind to E-selectin are increased in frequency in the blood and skin lesion of AD patients. These results indicate that preferential accumulation of CLA⁺ Th2 cells in the circulation is not a simple consequence of their intrinsically lower capacity to bind E-selectin (18,19). Thus, this subset is unlikely to represent T cells that have participated in local inflammatory responses but been then excluded from the effector site because of their inability to bind E-selectin upon the resolution of inflammation, because the terminally differentiated Fuc-TVII[−] CLA⁺ T cell incapable of binding E-selectin were not significantly increased in the blood of AD. The

Table 1. Expression of fucosyltransferase VII (Fuc-TVII) and cutaneous lymphocyte antigen (CLA) by infiltrating T cells in skin biopsy specimens of atopic dermatitis (AD) and psoriasis (Pso) lesions

	Fuc-TVII ⁺ CLA ⁺ /CLA ⁺ ¹		Fuc-TVII ⁺ CLA ⁺ /CLA ⁺ ²		Fuc-TVII ⁺ /CD3 ⁺ ³	
	AD	Pso	AD	Pso	AD	Pso
Epidermis	58.6 ± 5.3 ⁴	37.1 ± 7.0 ⁴	41.5 ± 5.2 ⁴	62.8 ± 7.0 ⁴	74.7 ± 6.4 ⁵	53.4 ± 5.9 ⁵
Dermis	51.8 ± 4.3 ⁵	15.2 ± 3.4 ⁵	47.5 ± 3.5 ⁵	84.9 ± 3.3 ⁵	36.2 ± 4.0 ⁴	17.2 ± 2.2 ⁴

¹Percentages of Fuc-TVII-positive cells in CLA-positive T cells.

²Percentages of Fuc-TVII negative cells in CLA-positive T cells.

³Percentages of Fuc-TVII positive cells in CD3-positive T cells.

⁴The Student's *t*-test was used to determine the significance of differences in samples.

Difference between AD and psoriasis is statistically significant (*P* < 0.05).

⁵Difference between AD and psoriasis is statistically significant (*P* < 0.01).

Fuc-TVII⁺ CLA⁺ subset most abundantly identified in the circulation of AD patients would include cells that were primed in lymph nodes draining the skin sites, and have circulated back to seed the skin. If this is true, this expanded population would be predictive of disease progression. Indeed, the present study shows that the frequencies of Fuc-TVII⁺ CLA⁺ or CCR4⁺ subset with the potent ability to bind E-selectin dramatically fell upon the improvement in symptoms. The selective increase in the Fuc-TVII⁺ ESL⁺ CLA⁺ Th2 cell population in the blood from AD patients could now be interpreted as indicating an expansion of skin-homing Th2 cells with highly potent ability to migrate into the skin, although the possibility remained to be excluded that CCR4 is also expressed on some Th1 cells, as demonstrated previously (28).

The frequencies of this Fuc-TVII⁺ CLA⁺ or ESL⁺ CLA⁺ phenotype in the blood are normally low as compared with the Fuc-TVII⁺ CLA⁺ phenotype because of a rapid cutaneous extravasation of this subset mediated by their potent ability to bind E-selectin. Given our findings that the vast majority of CLA⁺ CD4⁺ T cells able to bind to E-selectin express CCR4 and that there is a relationship between the increase in this population and the severity of clinical symptoms, it is a logical extension that additional migration of skin-homing Th2 cells with highly potent ability to migrate into the skin should be turned off at the peak of an immune response in order to protect the host from excessive immune stimulation, which could in itself lead to great pathological consequences. The regulatory requirement that determines when T-cell migration is turned off is a largely unappreciated and unexplained aspect of T cells. In this regard, our preliminary observations that circulating soluble E-selectin levels are increased in some of the deteriorating AD we investigated, in which the frequencies of Fuc-TVII⁺ CLA⁺ CD4⁺ T cells were markedly high, suggest that soluble selectins and chemokines may have an important regulatory influence on these processes, although highly speculative in this aspect.

Unexpected in our study was the observations that the vast majority of T cells found in the epidermis of AD patients expressed both Fuc-TVII and CLA and that the frequencies were significantly higher than that in psoriasis, which is characterized by Th1 cell infiltration (29). Recent studies (30) and our *in vitro* ongoing studies demonstrate that this Fuc-TVII⁺ CLA⁺ phenotype can only be induced by stimulating Th2 cells in the presence of interleukin (IL)-12 (Takahashi R, unpublished data). Given such a requirement for IL-12 in the acquisition of this phenotype for Th2 cells, our data suggest that IL-12 produced by Langerhans cells, keratinocytes and macrophages (31) is a predominant mediator for the generation of this phenotype in AD; in this regard, bacterial (32) and viral infections frequently observed in AD skin would serve to increase this phenotype via increased IL-12 production. If so, the Fuc-TVII⁺ CLA⁺ T cells found in the epidermis of AD patients might reflect *de novo* induction. The potent ability of these epidermal cells to induce this phenotype in AD patients raises the possibility that the ability of these epidermal cells to produce IL-12 in AD may be superior to that in psoriasis.

Our findings illustrate the clinical importance of identifying skin-homing T cells with the potent capacity to migrate to the skin by analysing Fuc-TVII expression and E-selectin-binding ability in patients with AD, thereby improving overall patient management. In summary, our data emphasize the necessity to target the Fuc-TVII enzyme more than the CLA epitope when designing therapeutic strategies for AD.

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